

Bulletin of the Agricultural Chemical Society of Japan.

CONTENTS

| | |
|--|----|
| Teizo TAKAHASHI, Kin-ichiro SAKAGUCHI and Toshinobu ASAI: On the Physiology of Rhizopus Species. | 87 |
| Yukihiko NAKAMURA: On the Differences of Brewing Barley according to Species. | 92 |
| II. The Kinetics of the Enzymatic Decomposition of the Proteins. | |
| Etsuo TAKAMIYA: On the Influence of Magnesium and Mangan-salts' Alcoholic Solutions upon the Indicator Phenolphthalein. | 94 |
| Masuzo SHIKATA & Isamu TACHI: Research on the Electrolytic Reduction Potentials of Organic Compounds. | 95 |
| Part III. Reduction Potentials of Nicotinic Acid. | |
| Kenjiro SHOJI: The Polarographic studies on the Fermentation Products. Part I | 96 |
| Sator OHDAKE: On the Chemical Constituents of Yeast-Extract. | 98 |

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President : Umetarō SUZUKI.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2. No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Editor: Umetarō SUZUKI.

Associate Editors: Kakuji GOTŌ and Yoshihiko MATSUYAMA.

ON THE PHYSIOLOGY OF RHIZOPUS SPECIES.

By TEIZO TAKAHASHI, KIN-ICHIRO SAKAGUCHI and
TOSHINOBU ASAI.

(Received June 20th., 1927.)

- PART. V. The preliminary research on the occurrence of zymase and carboxylase in Rhizopus species.
PART. VI. The verification of the occurrence of zymase in Rhizopus species.
PART. VII. On the formation of ethyl alcohol from acetic acid by acetone-Rhizopus (Rhizopus treated by acetone.)
PART. VIII. On the formation of ethyl alcohol from malic acid by Rhizopus species.
PART. IX. On the formation of ethyl alcohol from malic acid by acetone-Rhizopus.

As it is well known, that Rhizopus species, as a rule, play very important role in many kinds of the manufacture of alcoholic beverages, in saccharifying as well as in the formation of alcohol, we have a good reason to assume the existence of zymase and carboxylase in the fungus. Nevertheless, the confirmation of their existence lacked ever since. The authors devise to verify the occurrence of both zymase and carboxylase in the fungus foiled in the case when they allowed to follow the method of E. Buchner, as schemed in the case of yeast zymase. By the determination of alcohol produced by acetone-Rhizopus viz :- fungus growth treated by acetone, we could safely conclude the presence of zymase in this fungus.

The existence of carboxylase could not be confirmed yet, not only in the pressed juice of fungus, but also in acetone-Rhizopus.

The production of alcohol from acetic acid was affirmed, although the quantity of alcohol produced was not satisfactory great, by the dead cells of the fungus. There must exist some substance of an enzymic nature.

An analogous phenomenon was perceived in the formation of alcohol from malic acid either by fungus itself or by acetone-Rhizopus viz :- the dead cells.

From these data together with all the facts, which the authors have substantiated already, this fungus produces alcohol from many organic acids, such as acetic-, malic-, tartaric-, fumaric-, and gluconic acids and especially from the first two acids, alcohol formation is ascertained even by the action of the dead cells namely by special enzymes.

The evolution of CO₂ from organic acids by yeast or zymin is already

affirmed by C. Neuberg and L. Tir⁽¹⁾ and it will be perhaps the same nature of ours, although their aim have been to prove the occurrence of carboxylase.

However, if pyruvic acid is only intermediate, nearest to alcohol, product of ethyl alcohol formation, these alcohol giving acids must always and decidedly derived to their ends to pyruvic acid. For the derivation or degradation of these organic acids to pyruvic acid, therefore there must come into existence many enzymes, perhaps for each stage of the degradations. The researches for these enzymes will be followed hereafter.

The Experimental to PART V.

I. RESEARCH ON ZYMASE.

Fungus used :- Rhizopus oryzae.

The culture was obtained from "koji" extract (12°B), as culture medium, during 10 days at $25\text{--}30^{\circ}\text{C}$.

A). The preparation of pressed juice and research for zymase.

100g. of the culture of fungus was washed several times with distilled water and the mass of fungus was chopped into small pieces with sterile scissors, provided the treatment of the mass between sterile filter papers to remove water off as much as possible. To these fine pieces of the fungus, 100g. of fine silver sand and 20g. of diatom earth were mixed and in a porcelain mortor the mixture was ground until it changed into a consistency of dough. The doughy mass was wrapped in a silk cloth (Habutai), and submitted to a pressure of hand press to press out the juice, which attained about 30c.c. in the first pressing, to which added another 20c.c. of it in the second pressing, provided the addition of water and regrinding before the management.

The juice thus gained was introduced into Einhorn's fermenting tube with addition of glucose and antiseptic, to observe how the evolution of carbon dioxide comes forth, as tabulated below :-

- 1) Pressed juice 10 c.e. + glucose 20 % + toluol 0.1 c.c.
- 2) Pressed juice 10 c.e. + glucose 40 % + toluol 0.1 c.c.
- 3) Pressed juice 10 c.e. + glucose 20 % + toluol 0.1 c.c.
+ Buffer mixture with phosphate.
- 4) Pressed juice 10 c.e. + toluol 0.1 c.c.
- 5) Glucose (20%) 10 c.e. + toluol 0.1 c.c.

After 42 hours at $25\text{--}30^{\circ}\text{C}$, we could observe just a trace of gas evolved, which was almost equal to that of control (No. 4. in the table).

Thus we failed to prove the occurrence of zymase, but a little improvement of the instrument to collect the carbonic acid evolved to its last trace,

there came out the decisive positive prove of zymase. It will be reported before long.

B). The preparation of acetone-Rhizopus viz : fungus growth treated by acetone and research on zymase.

The preparate of acetone-Rhizopus was made by quite the same method as done in the case of acetone-yeast (zymin), after Albert, and its fermenting power was tested with Einhorn's tube as shown in the case of pressed juice. (refer heading A.) The results are tabulated below :-

| | | | Gas evolved after 42 hours at 25°-30°C. |
|----|-----------------|---|--|
| 1) | Glucose (20%). | 10 c.c. dead fungus 0.5 g. toluol 0.1 c.c. | Almost none |
| 2) | Glucose (40%). | 10 c.c. dead fungus 0.5 g. toluol 0.1 c.c. | Almost none |
| 3) | Glucose (20%). | 10 c.c. dead fungus 0.5 g. toluol 0.1 c.c. + buffer mixture with phosphate. | Almost none |
| 4) | Glucose (20%) | 10 c.c. + toluol 0.1 c.c. | none |
| 5) | Distilled water | 10 c.c. + dead fungus 0.5 g. + toluol 0.1 c.c. | none |

II. RESEARCH ON CARBOXYLASE.

The pressed juice of the fungus and the mentioned acetone-Rhizopus were tested for carboxylase in Einhorn's tube in analogous way to that of for zymase. The data are tabulated below :-

A). Pressed juice.

| | | Gas evolved at 25°- 30°C, aft. 18 hours. |
|-----|--|---|
| 1) | Pyruvic acid (1%) | 5c.c. + pressed juice 5c.c. + toluol 0.1c.c. Almost none |
| 2)* | Pyruvic acid (1%) $\{K_2HPO_4\}$ (1.5%) | 5c.c. + pressed juice 5c.c. + toluol 0.1c.c. Almost none |
| 3) | Pyruvic acid(0.5%) | 10c.c. + toluol 0.1c.c. none |
| 4) | Distilled water | 5c.c. + pressed juice 5c.c. + toluol 0.1c.c. none |

B). Acetone-Rhizopus.

| | | Gas evolved at 25°- 30°C, after 18 hours. |
|--------------------|---|---|
| 1) | Pyruvic acid (0.5%) | 10c.c. + fungus 0.5g. + toluol 0.1c.c. about 02.c.c. |
| 2)(²) | Pyruvic acid (0.5%) $\{K_2HPO_4\}$ (0.75%) | 10c.c. + fungus 0.5g. + toluol 0.1c.c. about 0.2c.c. |
| 3) | Pyruvic acid (0.5%) | 10c.c. + toluol 0.1c.c. none |
| 4) | Distilled water | 10c.c. + fungus 0.5g. + toluol 0.1c.c. none |

Thus, the authors were unable to substantiate the occurrence of carboxylase in Rhizopus, but they have very fine reason to be so and that will be shown later on.

* PH of the mixture was 3.5.

2) PH of the mixture was 3.5.

PART VI.

The verification of the occurrence of zymase :- The formation of alcohol from glucose by the said acetone-Rhizopus.

Fungus :- Rhizopus G. 34. Culture medium :- 500c.c. of Koji extract.
 + 25g. sucrose + 10g. CaCO₃. 10 days culture at 25°C.

The preparation of the durable fungus (acetone-Rhizopus) was quite same as described in part V, but the apparatus was changed to common flask with the aim to determine alcohol formed at the end of the test, provided with all means suitable for an aseptic working. The results are described in the table as follows :-

| | PH | Glucose 10g. $\frac{n}{15}$ KH ₂ PO ₄ 9c.c. $\frac{n}{15}$ Na ₂ HPO ₄ 1c.c. } 100c.c. | After 40 h ^s at 25-30°C. | | |
|----|---|--|-------------------------------------|----------------------------|-------------------|
| | | | Alcohol decomposed. v. % | Glucose in 100c.c. (g.) | remained. (g.) |
| A) | 5.3 | + 5g. dead fungus. | 0.20 | 1.26 | 8.74 |
| B) | " | " | boiled 2. h ^s | 0.07 | 0.89 |
| C) | Distilled water 90c.c. $\frac{n}{15}$ KH ₂ PO ₄ 9c.c. $\frac{n}{15}$ Na ₂ HPO ₄ 1c.c. } 100c.c. | 5.3 | + dead fungus 5g. reaction positive | Iodform | — |

From this, the authors are inclined to conclude that Rhizopus species contain an enzyme by whose action ethyl alcohol is formed from glucose and it may be called zymase defined by Ed. Buchner.

PART VII.

The formation of ethyl alcohol from acetic acid by acetone-Rhizopus.

Rhizopus species, culture medium and conditions for growth of the fungus are quite same as described in part VI. The results are shown in the table below :-

| | PH | Dead fungus | After 82 h. s. at 25-30°C. | | |
|----|---|--------------------|---------------------------------|---------------------|----------------------|
| | | | Alcohol v. % | decomposed. (g.) | Acetic acid. (g.) |
| A) | $\frac{n}{5}$ Acetic acid 15c.c. $\frac{n}{5}$ Na-acetate 75c.c. $\frac{n}{5}$ K ₂ HPO ₄ 10c.c. } 100c.c. | 5.2 ⁽³⁾ | 5g. | 0.13 | 0.3487 |
| B) | " | " | boiled 2 hours | 0.07 | 0.2647 |
| C) | Distilled water 90c.c. $\frac{n}{15}$ KH ₂ PO ₄ 9c.c. $\frac{n}{15}$ Na ₂ HPO ₄ 1c.c. } 100c.c. | 5.8 | Iodform reaction positive | — | — |

The authors could not deny the occurrence of an enzyme in Rhizopus species, which play an important rôle for the formation of alcohol from acetic acid.

3) It correspond to 1.0807 g. in 100 c.c. water.

PART VIII.

The formation of ethyl alcohol from malic acid by Rhizopus species.

Rhizopus sp. :- Rhizopus G. 34. Culture medium :- Koji-extract (6°B) + 5% of glucose

After 10 days culture in this medium at 28–30°C, the growth of fungus covered whole the surface of the medium. At this stage, the culture medium under the growth was decanted and washing several times with the sterile distilled water, until there remains any indication of the presence of acids, ethyl alcohol and sugars, culture media (a, b and c) anew was introduced from the side tube. (refer to Bulletin of Agr. Chem. Soc. Jap., Vol. 3. No. 3. p. 39) After 50 hours at 28–31°C., alcohol produced was determined, and in the meanwhile the fungus mass was held immersed under the fluid. The results are tabulated below :–

| Culture media | Weight of fungus. g. (dry matter) | PH | Acidity. | | Alcohol. (in 100c.c.) | Aldehyde (Schiff's reaction). |
|---|---|-----|---|-------------------------|---------------------------|-------------------------------------|
| | | | c.c. of N/10 NaOH to neutralize 5c.c. solut. | Original. After growth. | | |
| a) 1.5g. malic acid (merk), 0.5 KH ₂ PO ₄ , 1g. K ₂ HPO ₄ , 300c.c. water | 0.915 | 4.3 | 4.9 | 3.8 | 0.053g. | Positive. |
| b) 1.5g. malic acid, 300c.c. water | 0.873 | 3.0 | 4.0 | 3.6 | Iodform test positive. | Positive. |
| c) 0.5g. KH ₂ PO ₄ , 1g. K ₂ HPO ₄ , 300c.c. water. | 0.899 | 4.9 | — | — | Precip. of iodform. | Positive. |

Remarks :- At the end of the experiment, by the microscopic examination it was confirmed that the procedure had been going on in aseptic way.

PART IX.

The formation of ethyl alcohol from malic acid by acetone-Rhizopus.

The fungus used in this experiment was Rhizopus G. 34 cultured as noted in the part VI and the preparation of acetone-Rhizopus was quite same described in that part.

The dead fungus viz. acetone-Rhizopus was put into the special flasks as shown in a former paper⁽⁴⁾ and adding media (a, b, c), respectively to each flask, held at 28–30°C during 50 hours, provided all aseptic control is concerned. At the end of the experiment the decrease by weight of flask, including contents, was taken as the weight of carbon dioxide evolved in the time. The last trace of carbon dioxide was driven off by passing a current of air, free from moisture and carbon dioxide, warming to about 60°C the contents during the management. The results are indicated in the table below :–

4) Bulletin of the Agric. Chem. Soc. of Japan. Vol. 3. No. 3.

| Culture media | Weight of fungus(g.) (Acetone-Rhiz.) | pH | Alcohol | | CO ₂ (g.) | Malic Acid. | | |
|---|--|-----|-----------------|-----------------|-------------------------|-------------------------------|--------------------------------|----------------------------------|
| | | | g. in 70c.c. | w. % | | Applied. in 70c.c. (g.) | Remained. in 70c.c. (g.) | Decomposed. in 70c.c. (g.) |
| a) 0.6872g. malic acid, 100c.c. water. | 0.7g. Na ₂ HPO ₄ 12H ₂ O, | 1.5 | 4.6 | 0.077 | 0.11 | 0.0435 | 0.481 | 0.352 |
| b) Do | Do | 1.5 | 4.6 | 0.084 | 0.12 | 0.037 | 0.481 | 0.366 |
| c) 0.6872g. malic acid, 100c.c. water. | 0.7g. Na ₂ HPO ₄ 12H ₂ O, 0.4g. KH ₂ PO ₄ , 100c.c. water. | 1.5 | 5.2 | 0.021 (0.03) | — | 0.0105 | — | — |
| d) 0.6872g. malic acid, 100c.c. water. | Do | — | — | — | — | 0.007 (ave.) | 0.481 | — |

Remarks :- In a) and b) we observed in media crystals, very similar to that of fumaric acid, and it is rather out of question that the formation of the acid from malic acid happened by an enzyme specific for it. It will be substantiated in a near future.

(This paper was read already in the meeting of the Agricultural Chemieal Society of Japan, on February, 1927)

ON THE DIFFERENCES OF BREWING BARLEY ACCORDING TO SPECIES.

II. THE KINETICS OF THE ENZYMATICAL DECOMPOSITION OF THE PROTEINS

By YUKIHIKO NAKAMURA.

(Received July 16th., 1927.)

INTRODUCTION.

Attempting to study the differences of brewing barley according to the species, the author published his first report stating the physico-chemical differences of the proteins. The present investigation was carried out to study the kinetics of the enzymatic decomposition of the protein for the purpose of getting a partial knowledge concerning the constitution of the protein molecules.

The fact that an enzymatic decomposition of a protein has a relation to its constitution was accepted by many investigators, and in recent days, Ssadikow, Waldschmidt-Leitz and Sörensen have also published the same idea in their works.

EXPERIMENTS.

From three species of barley, -Golden Melon, Chevalier and Hokudai No. 1,- produced in the 11th, 12th and 13th years of Taisho, the author prepared proteins soluble in 10 % NaCl solution, in 70 % alcohol and in 0.2 % NaOH solution respectively. To the protein were added Sörensen's M/15 phosphate mixture of the Ph value 7.731 and a 1/7000 water solution of trypsin (made by Grübler). And also to the protein were added the mixtre of M/5 HCl and M/5 KCl (Ph value 1.2) and a 1/7000 water solution of pepsin (made by Merck). The mixtures were kept in an incubator at $40^{\circ} \pm 0.5^{\circ}\text{C}$. for 0, 15, 30, 45, 60, 90, 120, 150, 180 and 210 minutes, then the undigested protein was precipitated by an addition of the trichloroacetic acid solution. Taking the mixture of 0 minute's enzymatic action as a standard, a nephelometric comparison was carried out by means of a Duboscq's nephelometric colorimeter.

DISCUSSION AND CONCLUSION.

To make his experiments coincide with the equation as exactly as possible, the author has combined the equation of equi-bimolecular reaction

$$k = \frac{1}{t} \cdot \frac{a}{a(a-x)}$$

and Schütz's law $x = k \text{ pt}$,

and obtained the following equation;

$$k = \frac{1}{t^{k'}} \cdot \frac{a}{a(a-x)}$$

By the experiments and calculation according to the equation, the author was able to discuss and conclude as follows.

(1) By the method of least squares, the two constants k and k' of the above equation were calculated from the experimental data of trypsin and pepsin. The calculated values coincide very well with the experiments. This fact shows the correctness of the author's equation. That is, the decomposition of the proteins of brewing barley by trypsin and pepsin was expressed by the author's equation very well.

(2) In the case of trypsin, if k' is taken as the abscissa, and $\log k$ as the ordinates, the proteins soluble in 10% NaCl solution, in 70% alcohol, and in 0.2% NaOH solution make three straight lines respectively. Therefore, the relation between k and k' has to be expressed by the equation.

$$\log k = mk' + b.$$

The two constants of the straight line were calculated by the method of least squares. The two intersections of the three straight lines were

calculated as follows; (0.5309, -3.81421) and (0.9495, -4.97407).

(3) That the intersection were obtained shows that the three proteins, at least those in the barley, have some continuous constitution. It also seems that the three proteins gradually merge with each other either qualitatively or quantitatively taking as the limit the proteins whose decompositions are expressed by the curves made by the intersections.

According to the solubility of the proteins in the 10% NaCl solution, in the 70% alcohol or in the 0.2% NaOH solution, no proteins exceed the author's so-called limiting proteins. These facts which seem to prove the author's opinion concerning the constitution of the proteins deserve careful attention.

(4) The values of k seem to have some relation with the iso-electric point of the proteins. k seems to increase according to the increase of the PH value of the iso-electric point of the point of the proteins.

(5) The values of k' at the intersections of the three straight lines were 0.5309 and 0.9495 which approximate 0.5 and 1.0. If it as 0.5, the equation take on the form of Schütz's law, and if 1.0, the equation become like the form of the bimolecular reaction. Many investigators have already proved the correctness of Schütz's law or the bimolecular equation. Thus it seems that the investigators had limited their results to a specific protein or to the author's limiting proteins. Therefore, the author's limiting proteins had to resemble, at least on the relation to the action of the enzyme, the typical and the representative proteins investigated up to recent days. To expect a general constitution between the two kinds of proteins seems to be the most proper.

ON THE INFLUENCE OF MAGNESIUM-AND MANGAN-SALTS' ALCOHOLIC SOLUTIONS UPON THE INDICATOR PHENOLPHTHALEIN.

By ETSUO TAKAMIYA.

(Received July 5th, 1927.)

I reported the fact in the preceding paper "Experimental studies upon the alkalimetric estimation of amino acids and peptides by the method of Willstätter and Waldschmidt-Leitz" in this journal that Mg-chloride, Mg-sulphate, Mg-nitrate, Mn-chloride and Mn-sulphate behave as acid against

phenolphthalein in their alcoholic solutions.

Further studies on this problem resulted as follows.

1). This phenomenon is entirely due to the influence of these salts upon the indicator, phenolphthalein, in the presence of alcohols.

2). From the fact that this phenomenon occurs also in the case of thymolphthalein as well as of phenolphthalein, I suppose that the cause of this phenomenon is probably due to phthalein-group.

3). The degree of the influence of these salts upon phenolphthalein varies according to the concentration of alcohol.

4). At a definite concentration of alcohol the degree of the influence is proportional to a quantity of these salts present.

(Biochemical Laboratory, Department of Agriculture,
Kyushu Imperial University, Fukuoka.)

RESEARCH ON THE ELECTROLYTIC REDUCTION POTENTIALS OF ORGANIC COMPOUNDS.

PART III. REDUCTION POTENTIALS OF NICOTINIC ACID.

By MASUZO SHIKATA and ISAMU TACHI.

(Received Aug. 26th., 1927.)

Summary of the result:

(1) The reduction potential of nicotinic acid was measured with the dropping mercury cathode and the polarograph.

(2) Two stages of the reduction process were observed.

(3) For the first stage of reduction, observed R-P. was compared with the theoretical value calculated by the following formula at 15°C.,

$$\pi = - \frac{0.05713}{2} \log \frac{k'}{[H^+]^2 C_{C_5H_4N.COOH}}$$

in which by taking the R. P. of 0.01 mol nicotinic acid in (0.01n HCl + 0.1n KCl) solution, i. e. -0.984V as a standard, we have

$$\log k = 28.33$$

In an acidic as well as in a neutral salt solution, the observed R. P. showed satisfactory concordance with the calculated values, and thus the first stage has been concluded to be the reduction of the carbonyl group of nicotinic acid to aldehyde.

(4) The second stage of reduction is considered to be the reduction

of pyridine ring of nicotinic acid, by comparison with the R. P. of pyridine in our preceding paper.

(5) The reduction of nicotinic acid in an excess of alkali, does not take place, owing, perhaps, to the desorption of negatively charged nicotinic acid ions to the polarised mercury cathode. R. P. in a sodium acetate or sodium bicarbonate solution was about 0.02V more negative than the calculated value.

(6) Maximum of current voltage curve was observed in sodium bicarbonate solution, in which the potential of maximum current intensity was almost independent of the concentration of nicotinic acid.

(7) The reduction potential of benzoic acid was studied for the sake of comparison, with the result that in hydrochloric acid, no reduction but only the deposition of hydrogen ion has been seen, while a potassium chloride solution the R. P. is over 0.240V negative than that of nicotinic acid. Thus the group effect of a carbonyl group is considered to be more effective than a benzene ring.

(8) The decisive conclusion as to the reduction process has been left out for the time, when the isolation of the reduction product, now under investigation, would be completed.

THE POLAROGRAPHIC STUDIES ON THE FERMENTATION PRODUCTS. PART 1.

By KENJIRO SHOJI.

(Received Aug. 31st., 1927.)

Summary of results.

(1) The polarographic method has been applied as the qualitative as well as the quantitative microanalysis on the studies of reducible compounds in the fermentation products, such as "sake" (japanese rice wine), "shoyu" (soya bean sauce), wine, beer and commercial alcohol.

(2) For the "sake," we have found five reducible compounds, which are distinguishable by this method, from the reduction potentials and the saturation curves of polarograms.

They are

| | Reduction potential in 0.1 n NH_4Cl from N calomel electrode |
|-------------|---|
| Compound I | -0.22 V |
| Compound II | -0.40 V |

| | | |
|--------------|--|---------|
| Compound III | | -0.90 V |
| Compound IV | | -1.33 V |
| Compound V | | -1.63 V |

The reduction potentials of reducible substances possibly present in the sake are measured

| | | |
|-------------------|--------|--------------------------------------|
| Cinnamic aldehyde | 0.001% | -0.913 V in 0.1 n NH ₄ Cl |
| Furfural | 0.001% | -1.302 V in 0.1 n NH ₄ Cl |
| Acetaldehyde | 0.001% | -1.603 V in 0.1 n NH ₄ Cl |

Compound IV and V are found in the distillate (80°-100°C), while componnd I and II are observed in the residual solution after distillation ; compound III is found in both cases. Thus it is concluded that the compound III corresponds to the aromatic aldehyde, most probably cinnamic aldehyde or benzaldehyde, compound IV to be furfural, compound V to be aliphatic aldehyde, most probably acetaldehyde.

(3) In "shoyu", compound I, II, III, IV and V are found, in which compound II is much conspicuous than all the other cases.

(4) In wine, five compounds are also found ; but except comp-II, the waves are smaller than in the case of "sake".

(5) With respect to reducible compounds, beer is much simpler, and comp-III, IV and V only are recognisable, but not comp-I and II.

(6) In the commercial alcohol, reducible substance are also found, although much less in quantity, so it has been suggested, that this method can probably useful as one of the criterion for the purity of alcohol.

(7) Consideration has been given for the quantitative analysis of acet-aldehyde.

(8) Thus the polarographic method has proved to be of much applicable for the studies of the fermentation products, although this method is by no means the decisive identification of the reducible substances.

(In the Agricultural Chemical Laboratory,
Faculty of Agriculture, Kyoto Imperial University.)

ON THE CHEMICAL CONSTITUENTS OF YEAST-EXTRACT.

By Dr. SATOR OHDAKE.

Tokyo Imperial University.

(Received in August 27th., 1927)

The present communication deals with the chemical studies on the nitrogenous constituents of the yeast extract for the purpose of isolating the antineuritic substance in pure state. When the alcoholic extract of yeast is treated with a concentrated tannin solution, a precipitate is formed, which carries down a greater part of the active substance together with many other impurities. This tannin precipitate is dissolved in dilute acetone, decomposed with baryta water and filtered. When the filtrate is evaporated in vacuum after removing the excess of baryta, a brown resinous mass "tannin fraction" which possesses a strong antineuritic power, is obtained.

So, the author has started his studies with a large quantity of this tannin fraction, which was supplied from the Sankyo Company, Ltd., Tokyo, where "Oryzanin" or "Vitamin-B" -preparation is manufactured in large scale since 1912, under the supervision of Prof. Suzuki.

The tannin fraction was now separated into five fractions according to the method described below. The first four fractions (I--IV) were almost free from the antineuritic power, but the fifth one "Oryzanin fraction" was found to be highly active. The first four fractions were investigated thoroughly and the following substances were isolated in pure state, using 30,000kgs. of the pressed yeast as the material.

| | |
|--|---------|
| Adenyl-thiomethyl-pentose, $C_{11}H_{15}N_5SO_3$ | 480.0g. |
| Thioamino-Acid, $C_5H_{11}NSO_2$ | 0.6g. |
| Adenin | 206.0g. |
| Hypoxanthin | 31.8g. |
| Cholin | 15.4g. |
| Nicotinic acid | 26.0g. |
| Tyrosin | 0.3g. |
| Leucin | 25.8g. |
| Thymin | 9.0g. |
| Unknown base. I. $C_3H_6N_2$ | 2.0g. |
| Unknown base. II. $C_6H_8N_2O$ | 21.0g. |

Of these, Adenylthiomethyl-pentose⁽¹⁾ was discovered three years ago

1) U. Suzuki, S. Ohdake and T. Mori:- Journ. Agric. Chem. Soc. Japan, Vol. I, No. 2, p. 127—136, 1924; Biochem. Zeits. B. 154. H. 3—6, S. 278—289, 1924.

U. Suzuki and T. Mori:- Journ. Agric. Chem. Soc. Japan, Vol. I, No. 9, p. 653—661, 1925; Biochem. Zeit, B. 162. H. 3—6, S. 413—424, 1925.

by the author in association with U. Suzuki and T. Mori. The second sulphur-compound, thio-amino-acid,⁽¹⁾ was isolated by the author two years ago and was proved to be identical with the sulphur-compound isolated by J. H. Müller⁽²⁾ from the hydrolytic products of proteins. Nicotinic acid and Thymine were described by C. Funk.⁽³⁾

The constitution of the bases (I), $C_3H_6N_2$ and (II), $C_6H_8N_2O$ has not yet settled, but it was proved that they have no antineuritic power. Quite recently, a base having the formula $C_6H_{10}N_2O$ was isolated by Jansen and Donath⁽⁴⁾ from rice polishings and according to them, it possesses a strong antineuritic property. This compound is quite like the author's Base II and the difference between them is that the melting point of the hydro-chloride of the Base II is somewhat lower than that of the former and also no diazo-reaction with the Base II.

The fifth fraction "Oryzanin fraction" is now under investigation and the author hopes to be able to publish his further report in near future.

EXPERIMENTAL.

Fresh beer yeast, obtained from a brewery, was macerated several times with cold water, filtered with cloth, centrifuged, and pressed. The pressed yeast retaining still about 80% of water was then added with strong alcohol as much as to make alcoholic content 80% by volume, and after continual stirring for about 40 hours at room temperature, the mixture was pressed and filtered. The residue was once more extracted with 80% alcohol in the same manner. The united alcoholic extract was concentrated to a small volume. After removing the soluble matter by shaking with ether the light brown aqueous solution was further evaporated in vacuum to a syrupy consistency. In this way, the "alcoholic extract" containing about 20% of water was obtained. The yield was about 2.5% of the pressed yeast and its curative doses for pigeon was found to be 0.1—0.2g. per day.

The alcoholic extract thus prepared was now dissolved in twice of its volume of water and treated with a 20% aqueous solution of tannic acid, until no-more precipitate was produced. The voluminous precipitate thus obtained was now dissolved in dilute acetone, decomposed with baryta water and filtered. The filtrate freed from an excess of baryta was evaporated in vacuum to a small volume. On keeping for several days in a cool place,

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- 1) S. Ohdake:— Journ. Agric. Chem. Soc. Japan. Vol. I, No. 8, 1925. Biochem. Zeits. B. 161, H. 4—6, 1925. Journ. Agric. Chem. Soc. Japan. Vol. II, No. 10, 1926.
 - 2) J. H. Muller:— Journ. Bact. VII, 309—325, 1922. Journ. Biol. Chem. LVI. No. 1, 1923.
 - 3) C. Funk:— Journ. Physiol. 43, 395, 1911. Journ. Physiol. 45, 75, 1912. Journ. Physiol. 46, 173, 1913.
 - 4) B. C. P. Jansen and W. F. Donath:— Report of Med.-Labor. Weltevreden, Java, 1927.

spherical crystals separated out, which were collected, washed with a little cold water and dried; "Fraction I." The yield from 30,000 kgs. of pressed yeast amounted to 180g.

The mother liquor of the fraction (I) was again added with so much strong alcohol as to make its concentration of the solution about 80% by volume. A voluminous precipitate formed thereby was settled by keeping in a cool place, filtered by suction, washed with a small volume of 80% alcohol and dried. 120g. of brownish powder were thus obtained; "Fraction II."

The filtrate of the fraction (II) was concentrated in vacuum to a small volume and acetone was added as much as to make its content 50% by volume. The precipitate fromed thereby was filtered, washed carefully with dilute acetone and dried over sulphuric acid. About 130 gs. of a brown mass were obtained; "Fraction III."

The filtrate of the fraction (III.) was again evaporated in vacuum to a small volume and treated with absolute acetone. Whereby a greater part was thrown down as a brown resinous mass which possessed strong antineuritic property. It was dissolved in a small volume of water and evaporated in vacuum to a syrupy consistence containing about 15% of water. The curative dose of this fraction for pigeon was 0.01—0.015 per day. The yield was about 5% of the "alcoholic extract" or 0.125% of the original pressed yeast. To this fraction, the name "crude oryzanin" was given. The supernatant acetone solution displaying a light brown color was now concentrated in vaccum and 2,800 g. of dark brown syrup were obtained. It consisted chiefly of organic bases and resinous matter; "Fracion IV."

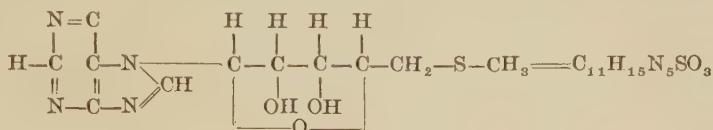
The author carried out thorough investigation on each fraction mentioned above and the results are described in the present communication. The "oryzanin-fraction" is now under examination, so it will be reported in the later opportunity.

FRACTION I.

The crude product designated as "Fraction I." was repeatedly crystallized from hot water and 70 g. of fine glistening colorless prisms were obtained, which were identified as adenylthiomethyl-pentose.

1) Adenylthiomethyl-pentose :- It melts at 209 - 210°C. (Uncorr.), and is readily soluble in hot water, but insoluble in alcohol and ether. Its specific rotatory power is: $[\alpha]_D^{17} = +20.1^\circ$, in 10% hydrochloric acid solution. It gives white precipitate with phosphotungstic acid or with phosphomolybdic acid. It gives also Kossel's and Bial's reactions as well as ferri-ferricyanide reaction. The sulphur in this compound is detectable either by sodium-nitroprusside or by lead acetate after boiling with strong alkali.

The analysis of the free base as well as of its picrate proved it to be a new sulphur-compound, having the empirical formulae $C_{11}H_{15}N_5SO_3$. When boiled with dilute acid, it is easily hydrolysed into adenin $C_5H_5N_5$ and a new thiosugar $C_6H_{12}SO_4$. Further studies showed its structure to be as follows:⁽¹⁾



From the mother liquor of adenylthiomethyl pentose about 30g. of potassium sulphate were obtained.

FRACTION II.

The "Fraction II" was dissolved in hot water, decolorized with charcoal, and while hot, about three times of its volume of absolute alcohol were added. Upon standing, fine spherical crystals separated, which after recrystallization from dilute alcohol, formed glistening colorless plates, melting at 285—287°C (Uncorr.) with decomposition. Yield: 15g.

[A] This compound resembled in its properties to leucin but it contained sulphur which was detectable either by sodium nitroprusside or by lead acetate after fusing with metallic sodium. Further studies showes it to be a mixture of leucin and a sulphur compound which were very difficult to separate each other by fractional crystallization. So it was dissolved in 300c.c. of water and treated with a hot saturated solution of mercuric chloride, whereby the sulphur compound alone, was precipitated leaving leucin in the solution.

2) Thioamino-acid $C_5H_{11}NSO_2$:- The white precipitate, obtained as above, was collected after standing over night and decomposed with hydrogen sulphide. The filtrate of mercury sulphide gave on evaporation, colorless crystals which were recrystallized from dilute alcohol. Yield: 0.6g.

It forms colorless, thin monoclinic plates melting at 271—272°C. (Uncorr.) with decomposition. It is readily soluble in water and dilute alcohol, but insoluble in ether, benzene, etc. Its specific rotatory power is $[\alpha]_D^{16} = -11.77^\circ$ in aqueous solution.

The empirical formula of this compound was proved to be $C_5H_{11}SNO_2$. It forms copper-salt $Cu(C_5H_{10}SNO_2)_2$, crystallizing in light blue thin monoclinic plates. The derivative of α -naphthylisocyanate $C_{16}H_{18}N_2SO_3$ crystallizes in white long needles, melting at 187°C. (Uncorr.) Further the β -naphthalene-sulpho-derivative ($C_{15}H_{17}S_2NO_4$) was prepared which crystallizes in white

1) U. Suzuki, S. Ohdake and T. Mori:- Journ. Agri. Chem. Soc. Japan. Vol. I, No. 2, 1924. Biochem. Zeits. B. 154, H. 3—6, 1924. U. Suzuki and T. Mori:- Journ. Agri. Chem. Soc. Japan. Vol. I, No. 9, 1925. Biochem. Zeits. B. 162, H. 3—6, 1925.

needles and melts at 204°C. (Uncorr.)

An aqueous solution of thioamino-acid gives a violet coloration with ninhydrin on warming, while Millon's, Folin's and biuret-reactions are all negative. With mercuric chloride, mercuric nitrate and mercuric sulphate, it gives a white precipitate, but it gives no precipitate either with phosphotungstic acid or with picric acid. Even a boiling strong alkali does not split sulphur from this compound. The nitroprusside and the lead-acetate reactions are only given when it is fused with metallic sodium. In contrary to ethyl-cystein, it is quite stable toward boiling strong alkali, giving neither ammonia nor ethylmercaptane.

From these properties, this compound was assumed to be a thioamino-acid having the formula $C_3H_7S-CHNH_2COOH$.⁽¹⁾ Recently, J. H. Müller⁽²⁾ isolated a sulphur-compound $C_5H_{11}SNO_2$ from hydrolytic products of casein. For the purpose of comparing these two compounds the present author prepared the same sulphur-compound from casein according to the Müller's mercuric method, and confirmed that it is identical with the thioamino-acid isolated from yeast-extract in all respect, except that the specific rotatory power of the former was little lower than the latter. It was found afterwards that, the Müller's compound was partially raceminized during the extraction with hot barya-water.

The author also isolated the same thioamino-acid from the hydrolytic product of yeast-protein by the same treatment, so its presence in the yeast extract might be due to the autolysis of yeast itself. As this compound was isolated, further, from egg-albumin, blood-fibrin and from the protein of rice-bran etc, it must be an important constituent of various protein.⁽³⁾

3) Leucin:— The filtrate from the mercuric precipitate of thioamino-acid was treated with hydrogen sulphide and evaporated in vacuum. The residue was dissolved in hot-water and treated with freshly prepared silver oxide to remove hydrochloric acid. The precipitate was filtered off and the filtrate was treated with hydrogen sulphide to remove the excess of silver and evaporated to a small volume. By adding twice of its volume of alcohol, leucin crystallized out forming glistening colorless thin plates which was recrystallized from hot dilute alcohol. Yield: 10.5g.

It melts and decomposes at 289—290°C (Uncorr.) in a sealed tube. It was dried at 100°C. in vacuo and analysed.

1) S. Ohdake:— Journ. Agric. Chem. Soc. Japan. Vol. I, No. 8, 1925. Biochem. Zeits. B. 161, H. 4—6, 1925.

2) J. H. Müller:— Journ. Bact. VII. 309—325, 1922. Journ. Biol. Chem. LVI. No. 1, 1923.

3) S. Ohdake:— Journ. Agric. Chem. Soc. Japan. Vol. II, No. 10, 1926.

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|-------------|------------------------|--------|
| 1 | 80.0 | 7.3 | 16 | 763 | 10.68 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 2 | 174.6 | 350.6 | 158.8 | 54.75 | 10.08 |

| Found | C% | H% | N% |
|--|-------|-------|-------|
| 1)..... | 54.75 | 10.08 | 10.68 |
| Calc. for C ₆ H ₁₂ NO ₂ | 54.97 | 9.99 | 10.69 |

[B] The filtrate from the mixture of the thioamino-acid and leucin was concentrated to about 500c.c., and precipitated with phosphotungstic acid. The precipitate was then decomposed with baryta. The filtrate of barium tungstate was evaporated to a small volume after removing the excess of baryta, and treated with an aqueous solution of silver nitrate.

(a) The precipitate thus obtained, was distributed in a little water and treated with ammonia. After standing over night, the silver precipitate was collected, suspended in water and decomposed with hydrogen sulphide. The filtrate from silver sulphide was concentrated to a small volume, made alkaline with ammonia and kept in a cold place when adenin separated out as fine crystals. It was recrystallized from hot water. Yield : 2.8g.

4) Adenin :- It forms microscopic short, white needles, sparingly soluble in water. Heated in a capillary, it darkens at 280°C. (Uncorr.) It gives Kossel's adenin reaction while Weidel's; Xanthin and diazo-reaction are all negative. It was dried at 100°C. in vacuo and analysed :-

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 2.035 | 0.867 | 0.915—2 Vol% | 16 | 762 | 52.20 |

Calculated for C₆H₅N₅ 51.85

Adenin picrate :- It crystallizes in long yellow needles, sparingly soluble in water.

Analysis of the picrate :-

Nitrogen :

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 2.49 | 0.652 | 0.665—2 Vol% | 16 | 756 | 30.76 |

Calculated for C₆H₅N₅—C₆H₅N₈O₇ 30.79

5) Hypoxanthin picrate :- When the mother liquor of adenin, was concentrated, neutralised with hydrochloric acid, and treated with sodium picrate, the crystals of hypoxanthin picrate were obtained. They were washed

with acetone and recrystallized from hot alcohol. Yield: 0.15g.

It forms light yellow, thick plates, melting at 247°C (Uncorr.), readily soluble in hot water, but insoluble in ether and benzene.

Microanalysis after Pregl :-

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-Pres. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|-----------------------|--------|
| 1 | 3.82 | 0.843 | 0.86—2Vol% | 17 | 770 | 26.32 |
| 2 | 5.17 | 1.137 | 1.16—2Vol% | 16 | 770 | 26.49 |

b) Carbon and hydrogen :

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 3 | 8.58 | 11.52 | 1.64 | 36.62 | 2.12 |

| | | | |
|---|-------|------|-------|
| Found/by Pregl's micro-analysis. | C% | H% | N% |
| 1)..... | 36.62 | 2.12 | 26.32 |
| 2)..... | — | — | 26.49 |
| Calculated for...C ₅ H ₄ N ₄ O-C ₆ H ₃ N ₃ O ₇ | 36.44 | 1.92 | 26.85 |

(b) The filtrate from the silver-precipitate (a) was freed from silver by means of hydrogen sulphide and evaporated in vacuum to expell off the ammonia and hydrogen sulphide, sulphuric acid was added to the extent of 5% of the solution and precipitated with phosphotungstic acid. The precipitate was decomposed with baryta in usual way and after removing the excess of baryta with sulphuric acid, the solution was concentrated in vacuum to a small volume whereby adenin nicotinate separated out as the aggregates of white needles.

6) Adenin nicotinate :- The crude product was recrystallized from hot water. Yield: 1g.

It forms white needles, aggregated in stelli-form, readily soluble in hot water, but sparingly in cold-water. It melts at 210—230°C. (Uncorr.) It gives Kossel's adenin reaction, while Xanthin, Weidel's and sulphur reactions are all negative.

From these properties this compound was assumed to be a salt of adenin and a substance of acidic nature. For the purpose of separating adenin from this compound, the latter was converted into picrates and subjected to fractional crystallization, whereby adenin picrate separated first from the aqueous solution, forming a long yellow needles. It was dried at 100°C in vacuo, and analysed.

Adenin picrate :-

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|--|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 3.03 | 0.789 | 0.805—2Vol% | 17 | 764 | 30.81 |
| Calculated for.....C ₅ H ₅ N ₅ -C ₆ H ₃ N ₃ O ₇ | | | | | | 30.79 |

Picrate of nicotinic acid :- The filtrate of adenin picrate gave on further concentration the picrate of nicotinic acid which was recrystallized from hot alcohol. Yield : 0.15g.

It forms light yellow thick plates, melting at 219°C. (Uncorr.) Dried at 100°C. in vacuum and analysed.

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|----------------------|---------------------------------------|--------------------|--------------------------|-------------|-----------------------|--------|
| 1 | 3.125 | 0.427 | 0.436—2 Vol% | 18.5 | 754 | 15.89 |
| Calculated for | <chem>C5H4NCO2H-C6H3N3O7</chem> | | | | | 15.91 |

The above figure agrees with the picrate of nicotinic acid, so the compound described under (6) was adenin nicotinate.

(c) The filtrate of the fraction (a), was treated with silver nitrate and baryta water. The precipitate formed thereby was suspended in water, decomposed with hydrogen sulphide and evaporated in vacuum. Sulphuric acid was added to the extent of 5% of the solution and precipitated with phosphotungstic acid. The precipitate was decomposed with baryta in usual way and concentrated in vacuo to a small volume. By adding picric acid a picrate of unknown base separated out as yellow plates which were filtered and recrystallized from dilute alcohol. Yield : 0.05g.

7) Picrate of unknown base. (C6H8N2O) :- Glistening light yellow plates, readily soluble in hot water and alcohol, but sparingly soluble in cold water, melts sharply at 192°C. (Uncorr.) without decompositon. After drying at 100°C in vacuo, it was analysed.

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|-----------------------|--------|
| 1 | 4.215 | 0.6566 | 0.67—2 Vol% | 17 | 767 | 18.50 |
| 2 | 4.465 | 0.7105 | 0.725—2 Vol% | 17 | 767 | 18.90 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % | N % |
|--|---------------|------------------------|-------------------------|--------|--------|--------|
| 3 | 7.14 | 10.80 | 1.84 | 41.25 | 2.86 | 18.50 |
| 4 | 7.20 | 10.93 | 1.89 | 41.40 | 2.92 | 18.90 |
| Found/by Pregl's micro-method. | | | C% | H% | N% | |
| 1) | | | 41.25 | 2.86 | 18.50 | |
| 2) | | | 41.40 | 2.92 | 18.90 | |
| Calculated for ... <chem>C6H8N2O-C6H3N3O7</chem> | | | 40.79 | 3.11 | 19.88 | |

Unfortunately, the material was not sufficient for further study but judged from its properties, it is probably the same compound with the base (No. 27).

8) Picrate of adenylthiomethyl-pentose :- The filtrate from the above picrate (7), gave on further concentration another picrate consisting of light

yellow plates, which after recrystallization from dilute alcohol, weighed 0.18g.

It melts at 165°C (Uncorr.) and gives strong sulphur reaction. Dried at 100°C in vacuo, and analysed.

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 4.600 | 0.8085 | 0.825-2Vol% | 16 | 768 | 20.98 |
| 2 | 4.355 | 0.7595 | 0.775-2Vol% | 16 | 768 | 20.81 |
| 3 | 3.11 | 0.5437 | 0.565-2Vol% | 14 | 762 | 20.85 |
| 4 | 3.54 | 0.6076 | 0.620-2Vol% | 14 | 764 | 20.52 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 5 | 7.575 | 10.67 | 2.61 | 38.54 | 3.83 |
| 6 | 7.170 | 10.05 | 2.42 | 38.23 | 3.75 |
| 7 | 7.30 | 10.04 | 2.47 | 37.51 | 3.75 |
| 8 | 7.36 | 10.19 | 2.31 | 37.76 | 3.49 |

Found/by Pregl's micro-method.

| | C % | H % | N % | S % |
|----------|--------|--------|--------|--------|
| 1)..... | 38.54 | 3.83 | 20.98 | — |
| 2)..... | 38.23 | 3.75 | 20.81 | — |
| 3)..... | 37.51 | 3.75 | 20.85 | — |
| 4) | 37.76 | 3.49 | 20.52 | — |

Calculated for ...C₁₁H₁₈N₅SO₃-C₆H₈N₃O₇ 38.78 3.42 21.29 6.08

The analysis agrees thus with the picrate of adenylthiomethyl-pentose.

(d) The filtrate from the fraction (c), was freed from silver and baryta by treating with hydrochloric and sulphuric acids, and precipitated with phosphotungstic acid. The precipitate thus formed was decomposed with baryta water. The filtrate, containing the free base was freed from baryta by sulphuric acid, concentrated in vacuo to a small volume and picric acid was added to it. On cooling cholin picrate separated out, which were recrystallized from hot dilute alcohol. Yield : 2.5g.

9) Cholin picrate :- Light yellow macroscopic prisms, readily soluble in water and melts at 245°C (Uncorr.). Dried at 100°C in vacuo and analysed :

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 6.02 | 0.823 | 0.84-2Vol% | 17 | 761 | 16.11 |
| 2 | 5.43 | 0.745 | 0.76-2Vol% | 17 | 755 | 16.04 |
| | | | | | | 16.00 |

Calculated for C₅H₁₈NO₂-C₆H₂(NO₂)₃OH

The above result agrees fairly with cholin picrate.

Chloro-aurate of cholin :- The chloro-aurate was prepared from the picrate. It forms characteristic orange needles, melting at 249°C (Uncorr.).

Dried at 100°C in vacuo and analysed :–

a) Nitrogen :–

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction, c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 8.46 | 0.235 | 0.24-2Vol% | 16 | 760 | 3.28 |
| 2 | 7.43 | 0.198 | 0.202-2Vol% | 16 | 760 | 3.15 |

b) Carbon and hydrogen :–

| No. | Subst. mg. | CO ₂ mg. | H ₂ mg. | Au mg. | C % | H % | Au % |
|-----|---------------|------------------------|-----------------------|-----------|--------|--------|---------|
| 3 | 9.11 | 4.56 | 2.89 | 4.035 | 13.65 | 3.52 | 44.29 |

| Found/by Pregl's micro-method | C % | H % | N % | Au % |
|---|--------|--------|--------|---------|
| 1)..... | 13.65 | 3.52 | 3.28 | 44.29 |
| 2)..... | — | — | 3.15 | — |
| Calculated for... C ₅ H ₁₄ NOCl AuCl ₃ | 13.54 | 3.16 | 3.16 | 44.47 |

[C.] The filtrate from phosphotungstic precipitate [B], was treated with a slight excess of barya and filtered. The filtrate was freed from an excess of barya and concentrated in vacuo to a small volume, upon standing white crystals of tyrosin separated out. They were recrystallized from hot water. Yield : 0.3g.

10) Tyrosin :– Long white needles, sparingly soluble in water. Gives an intense coloration with Millon's reagent and with ninhydrin. Dried at 100°C in vacuo and analysed :–

Nitrogen :–

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction, c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|---|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 5.43 | 0.363 | 0.37-2Vol% | 19 | 760 | 7.81 |
| 2 | 8.15 | 0.539 | 0.55-2Vol% | 19 | 760 | 7.73 |
| Calculated for..... C ₉ H ₁₁ (OH)C ₂ H ₃ NH ₂ COOH | | | | | | 7.74 |

11) Leucin :– The filtrate from tyrosin gave on further concentration 10.5g. of pure leucin.

It forms glistening plates, and decomposes at 289—293°C. (Uncorr.). Dried at 100°C in vacuo and analysed :–

a) Nitrogen :–

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correrection, c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|----------------------------|-------------|------------------------|--------|
| 1 | 7.33 | 0.666 | 0.68-2Vol% | 17 | 760 | 10.69 |

b) Carbon and hydrogen :–

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 2 | 8.73 | 17.53 | 7.90 | 54.76 | 10.05 |

| Found/by Pregl's micro-method. | C % | H % | N % |
|--|--------|--------|--------|
| 1)..... | 54.76 | 10.05 | 10.69 |
| Calculated for... C ₆ H ₁₃ NO ₂ | 54.97 | 9.99 | 10.69 |

FRACTION III.

The fraction III obtained by treating with 50% acetone, weighed about 130g. when washed with a small volume of 50% acetone and dried. It was dissolved in hot water, decolorized with charcoal, and the colorless crystals separating out on cooling, were recrystallized from hot water 80g. of pure adenylthiomethyl-pentose ($C_{11}H_{15}N_5SO_3$) were thus obtained.

12) Adenylthiomethyl-pentose :- Colorless prisms with silky lustre, melting at 209—210°C (Uncorr.) without decomposition, readily soluble in water, gives Kossel's, Bial's and sulphur reactions etc. Boiled with diluted acid, it is easily hydrolysed into adenin ($C_5H_5N_5$) and a thio-sugar ($C_6H_{12}SO_4$).⁽¹⁾

The mother liquor of adenylthiomethyl-pentose gave on further evaporation nothing but potassium sulphate.

FRACTION IV.

The brown syrup, fraction IV, gave after long keeping in a cool-place, spherical crystals of adenylthiomethyl-pentose. The yield of the crude product was about 400g. After recrystallization from hot-water, 310g. of pure adenylthiomethylpentose melting at 209—210°C (Uncorr.) were obtained.

13) Adenylthiomethyl-pentose :- As has already been mentioned, it gave, in addition to Kossel's adenin reaction and Bial's pentose reaction etc. an intensive sulphur reaction and was readily hydrolysed into adenin and a thio-sugar by boiling with dilute acid.

Concentrating further the filtrate from adenylthiomethylpentose, about 2.400g. of a syrup were obtained. It was dissolved in about eight times of its volume of hot water. The resinous substance separating on cooling was filtered off, and the filtrate was treated with basic lead acetate.

[A] The lead acetate precipitate was decomposed with hydrogen sulphide. The filtrate from lead sulphide was evaporated in vacuo and a dark-brown resinous mass was obtained. It was sparingly soluble in water but readily soluble in alcohol, acetone and alkalies. The same resinous substance was found always accompanied in every fractions coming next.

The filtrate of lead acetate precipitate was treated with sulphuric acid to remove the excess of lead and a 50% aqueous solution of phosphotungstic acid was added to it.

[B] The phosphotungstic acid precipitate was decomposed with baryta water in usual way and concentrated in vacuo to a small volume. On cooling,

1) U. Suzuki, S. Ohdake and T. Mori:- Journ. Agric. Chem. Soc. of Japan. Vol. I, No. 2, 1924. Biochem. Zeits. B. 154, Heft. 3—6, 1924. U. Suzuki and T. Mori:- Journ. Agric. Chem. Soc. of Japan. Vol. I, No. 9, 1925. Biochem. Zeits. B. 162. H. 3—6, 1925.

fine crystals of adenin separated out, which were recrystallized from hot-water. Yield : 75g.

14) White short needles, sparingly soluble in water, gives Kossel's adenin reaction, while Weidel's and Xanthin reactions etc. are all absent. Its picrate crystallizes in characteristic long yellow needles, sparingly soluble in water.

Dried at 100°C in vacuo and analysed :-

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 2.06 | 0.921 | 0.94—2 Vol% | 18 | 754 | 52.03 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 2 | 7.25 | 11.74 | 2.44 | 44.16 | 3.74 |

| Found/by | Pregl's micro-analysis. | C % | H % | N % |
|--|-------------------------|--------|--------|--------|
| 1) | | 44.16 | 3.74 | 52.03 |
| Calculated for... C ₅ H ₆ N ₅ | | 44.44 | 3.71 | 51.85 |

(a) The filtrate from adenin crystals was acidified with nitric acid and treated with 20% aqueous solution of silver nitrate. A voluminous precipitate formed, was collected and treated with ammonia to convert it into silver salt. After standing for 15hs., the insoluble silver salt was collected and decomposed with hydrogen sulphide. The filtrate gave on evaporation fine white crystals which were recrystallized from hot water. Yield: 66g.

15) Adenin-Hypoxanthin :- Short white needles, melted at 325—327°C (Uncorr.) and unlike adenin, it is readily soluble in hot water with neutral reaction. It gives also Kossel's reaction, but Weidel's, Xanthin- and diazo-reactions are all negative. From these properties, it was assumed to be adenin-hypoxanthin.⁽¹⁾

Dried at 100°C in vacuo and analysed.

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 2.25 | 0.897 | 0.915—2 Vol% | 17 | 759 | 46.84 |
| 2 | 2.05 | 0.804 | 0.82—2 Vol% | 17 | 764 | 46.40 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 3 | 7.90 | 12.84 | 2.92 | 44.33 | 4.08 |

1) Bruhs :- Zeits. f. Physiol. Chem. 14. 561. 1890.

| Found/by Pregl's microanalysis | C% | H% | N% |
|--|-------|------|-------|
| 1)..... | 44.33 | 4.08 | 46.84 |
| 2)..... | — | — | 46.40 |
| Calculated for ...C ₅ H ₅ N ₅ -C ₆ H ₈ N ₃ O | 44.28 | 3.82 | 46.50 |

The above result confirms the first assumption, so it was tried to separate these components by converting it into picrates. For this purpose 4g. of this compound were dissolved in water and treated with a slight excess of picric acid. The picrate of adenin separated first forming characteristic long needles. The yield of the purified picrate was 3g.

Nitrogen :-

| No. | Subst. | Vol. of N. | Vol-Correction. | Temp. | Atmosph-Press. | N |
|---------------------|---|------------|-----------------|-------|----------------|-------|
| | mg. | c.c. | c.c. | C. | mm. | % |
| 1 | 3.00 | 0.789 | 0.805-2Vol% | 17 | 760 | 30.96 |
| Calculated for..... | C ₅ H ₅ N ₅ -C ₆ H ₈ N ₃ O ₇ | | | | | 30.79 |

Hypoxanthin picrate :- The filtrate from adenin picrate was concentrated to a small volume and cooled. The crystals of hypoxanthin picrate separated as light yellow thick plates, melting at 255°C. (Uncorr.) Yield : 2g. Dried at 100°C in vacuo and analysed ;

a) Nitrogen :-

| No. | Subst. | Vol. of N. | Vol-Correction. | Temp. | Atmosph-Press. | N |
|-----|--------|------------|-----------------|-------|----------------|-------|
| | mg. | c.c. | c.c. | C. | mm. | % |
| 1 | 3.97 | 0.926 | 0.945-2Vol% | 17 | 760 | 27.45 |
| 2 | 4.06 | 0.921 | 0.94-2Vol% | 17 | 760 | 26.91 |
| 3 | 3.32 | 0.735 | 0.75-2Vol% | 18 | 756 | 26.72 |

b) Carbon and hydrogen :-

| No. | Subst. | CO ₂ | H ₂ O | C | H |
|-----|--------|-----------------|------------------|-------|------|
| | mg. | mg. | mg. | % | % |
| 4 | 7.91 | 10.56 | 1.77 | 36.41 | 2.49 |
| 5 | 7.10 | 9.47 | 1.41 | 36.25 | 2.21 |

| Found/by Pregl's micro-analysis | C% | H% | N% |
|--|-------|------|-------|
| 1)..... | 36.41 | 2.49 | 27.45 |
| 2)..... | 36.25 | 2.21 | 26.91 |
| 3)..... | — | — | 26.72 |
| Calculated for ...C ₅ H ₄ N ₄ O-C ₆ H ₈ N ₃ O ₇ | 36.44 | 1.92 | 26.85 |

16) Adenin picrate :- The mother liquor from the adenin-hypoxanthin was evaporated in vacuum to dryness, the residue was dissolved in hot-water and added with picric acid. Upon standing, adenin picrate separated out. After recrystallization from hot dilute alcohol, it weighed 60g.

It forms characteristic long yellow needles, sparingly soluble in water and melts at 290°C. (Uncorr.) with decomposition.

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|--|---------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 3.09 | 0.813 | 0.83-2Vol% | 17 | 760 | 30.97 |
| 2 | 2.91 | 0.764 | 0.78-2Vol% | 17 | 760 | 30.90 |
| Calculated for.....C ₅ H ₅ N ₅ -C ₆ H ₅ N ₃ O ₇ | | | | | | 30.79 |

17) Picrate of adenyl-thiomethyl-pentose :- When the mother liquor of adenin picrate was concentrated further and cooled, the picrate of adenyl-thiomethyl-pentose separated. Recrystallized from dilute alcohol, it formed light yellow thin plates, melting at 165°C. (Uncorr.) Yield : 9g.

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 3.68 | 0.671 | 0.685-2Vol% | 17 | 764 | 21.57 |
| 2 | 4.66 | 0.853 | 0.87-2Vol% | 17 | 756 | 21.43 |

b) Sulphur :-

| No. | Subst. mg. | BaSO ₄ mg. | S mg. | S % |
|--|---------------|--------------------------|----------|--------|
| 3 | 8.86 | 3.86 | 0.53143 | 6.05 |
| Found/by Pregl's microanalysis | | C% | H% | N% |
| 1) | — | — | — | 21.57 |
| 2) | — | — | — | 21.43 |
| Calculated for.....C ₁₁ H ₁₅ N ₅ SO ₃ -C ₆ H ₅ N ₃ O ₇ 38.78 | | 3.42 | 21.29 | 6.08 |

18) Hypoxanthin picrate :- The mother liquor from the above picrate (17) was further concentrated, the resinous matter thereby separated was removed by filtration. On keeping the filtrate in a cool-place, hypoxanthin picrate crystallized out as fine yellow thick plates which were recrystallized from hot dilute alcohol. Yield : 4.8g. M. p. = 247°C. (Uncorr.)

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 4.08 | 0.931 | 0.95-2Vol% | 17 | 760 | 26.86 |
| 2 | 3.99 | 0.911 | 0.93-2Vol% | 17 | 760 | 27.09 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|---|---------------|------------------------|-------------------------|--------|--------|
| 3 | 7.50 | 10.02 | 1.04 | 36.44 | 1.85 |
| Found/by Pregl's microanalysis | | C% | H% | N% | |
| 1) | — | 36.44 | 1.85 | 26.86 | |
| 2) | — | — | — | 27.09 | |
| Calculated for.....C ₅ H ₄ N ₄ O-C ₆ H ₅ N ₃ O ₇ | | 36.16 | 1.92 | 26.85 | |

(b) The ammoniacal filtrate from the insoluble silver salt (a) already mentioned, was treated with hydrogen sulphide and filtered off the silver

sulphide. To the filtrate baryta water was added in slight excess and evaporated in vacuum to expell off the ammonia, sulphuric acid was added to remove baryta and then precipitated with phosphotungstic acid. A voluminous precipitate formed thereby was decomposed with baryta water. The filtrate, freed from the excess of baryta was evaporated in vacuum to a small volume. Adenin separated as microscopic white crystals.

19) Adenin :- White short needles, sparingly soluble in water, and gives Kossel's adenin reaction but Weidel's, Xanthin-and diazo-reactions are negative. It was converted into picrate and analysed :

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|---------------------|--------------------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 3.12 | 0.8134 | 0.83-2Vol% | 17 | 764 | 30.85 |
| Calculated for..... | $C_5H_5N_5-C_6H_8N_3O_7$ | | | | | 30.79 |

20) Adenin nicotinate :- The filtrate from adenin was concentrated in vacuum to a small bulk. On cooling, the aggregates of white needles were obtained. Yield : 4.2g.

Although this compound gives intensive adenin reaction, yet it is not adenin itself because it dissolves easily in hot water and melts at 209-240°C. (Uncorr.) The analysis gave the following results :-

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 2.06 | 0.578 | 0.59-2Vol% | 17 | 754 | 32.76 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 2 | 7.23 | 14.51 | 3.17 | 54.73 | 4.87 |

| Found/by Pregl's microanalysis | C% | H% | N% |
|--|-------|------|-------|
| 1)..... | 52.85 | 4.10 | 32.76 |
| Calculated for $C_5H_4NCO_2-C_5H_5N_5$ | 51.36 | 3.50 | 32.68 |

From the above results it was assumed to be adenin-nicotinate. To confirm this assumption, it was converted into picrate and subjected to fractional crystallization, whereby adenin-picrate first crystallized out as long yellow needles.

Adenin picrate :-

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|---------------------|--------------------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 3.02 | 0.789 | 0.805-2Vol% | 17 | 764 | 30.91 |
| Calculated for..... | $C_5H_5N_5-C_6H_8N_3O_7$ | | | | | 30.79 |

Picrate of nicotinic acid :- The mother liquor of adenin-picrate, concent-

rated to a small volume, gave on cooling the picrate of nicotinic acid. Light yellow, thick plates, M. P.=219°C. (Uncorr.)

Analysis of the picrate :-

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 3.715 | 0.51 | 0.59-2Vol% | 17 | 754 | 15.69 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 2 | 7.89 | 11.92 | 1.75 | 41.20 | 2.34 |

| | | | | |
|----------------|--|-------|------|-------|
| Found/by | Pregl's micro-method | C% | H% | N% |
| 1) | | 41.20 | 2.34 | 15.69 |
| Calculated for | C ₅ H ₄ NCO ₂ H-C ₆ H ₃ N ₃ O ₇ | 41.19 | 2.27 | 15.91 |

From these results the above compound (20) was proved to be adenin-nicotinate.

21) Picrate of nicotinic acid :- To the mother liquor of adenin-nicotinate (20), picric acid was added, after a short standing, the picrate of nicotinic acid crystallized out light yellow, thick plates. M. P. 219°C. (Uncorr) Yield : 6g.

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|----------------|--|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 3.82 | 0.514 | 0.525-2Vol% | 17 | 754 | 15.71 |
| Calculated for | C ₅ H ₄ NCO ₂ H-C ₆ H ₃ N ₃ O ₇ | | | | | 15.91 |

(c) The filtrate from the purin-fraction (b), was neutralised with BaCO₃ and treated with a 20% aqueous solution of silver nitrate. The voluminous precipitate formed was filtered by suction and decomposed with hydrogen sulphide. The filtrate, thus obtained, was evaporated in vacuum to a small volume and after adding sulphuric acid to the extent of 5% of the solution, it was precipitated with phosphotungstic acid.

The precipitate formed thereby decomposed with baryta water and concentrated to a small volume. Upon standing, adenin separated as short white needles. Yield : 18.6g.

22) Adenin :-

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 2.00 | 0.887 | 0.905-2Vol% | 17 | 754 | 51.79 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | CO mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|-----------|--------|--------|
| 2 | 6.78 | 10.95 | 2.29 | 2.29 | 44.05 | 3.82 |

| | | | |
|---|-------|------|-------|
| Found/by Pregl's microanalysis | C% | H% | N% |
| 1)..... | 44.05 | 3.82 | 51.79 |
| Calculated for.....C ₆ H ₅ N ₅ | 44.44 | 3.71 | 51.85 |

23) Adenin nicotinate :- The filtrate from adenin was further concentrated and left in a cool place when white crystals of adenin nicotinate separated. Yield : 6g. White needles, aggregated in a stelli-form, M. P.=210–240°C (Uncorr.). Unlike adenin, it dissolves easily in hot water with slightly acid reaction, although it gives intensive adenin-reaction.

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|--|---------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 2.02 | 0.568 | 0.58-2Vol% | 17 | 754 | 32.83 |
| Calculated for.....C ₆ H ₅ N ₅ -C ₆ H ₄ CO ₂ | | | | | | 32.56 |

Adenin picrate :- The picrate was prepared from the above compound (23) and fractionally crystallized. Adenin picrate first separated as long yellow needles.

Analysis of adenin picrate :-

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|--|---------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 3.31 | 0.862 | 0.88-2Vol% | 17 | 760 | 30.65 |
| Calculated for.....C ₆ H ₅ N ₅ -C ₆ H ₃ N ₃ O ₇ | | | | | | 30.79 |

Picrate of nicotinic acid :- The picrate was obtained from the mother liquor of adenin picrate. M. P.=219°C. (Uncorr.)

Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|---|---------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 5.03 | 0.688 | 0.72-2Vol% | 17 | 761 | 16.12 |
| Calculated for.....C ₆ H ₄ NCO ₂ H-C ₆ H ₃ N ₃ O ₇ | | | | | | 15.91 |

The analysis agrees well with the picrate of nicotinic acid.

24) Picrate of adenylthiomethyl-pentose :- The filtrate from adenin-nicotinate (23) was treated with picric acid whereby the picrate of adenylthiomethyl-pentose separated in short yellow needles, which were recrystallized from dilute alcohol. Yield : 30g.

Microanalysis after Pregl :-

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol-Correction. c.c. | Temp. C. | Atmosph-Press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|-----------------------|--------|
| 1 | 4.95 | 0.965 | 0.985-2Vol% | 17 | 754 | 22.76 |
| 2 | 4.09 | 0.774 | 0.79-2Vol% | 17 | 764 | 22.64 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % | |
|---|---------------|------------------------|-------------------------|--------|--------|-------|
| 3 | 7.39 | 10.45 | 2.33 | 38.57 | 3.50 | |
| Found/by Pregl's micro-method | | | | C% | H% | N% |
| 1)..... | | | | 38.57 | 3.50 | 22.76 |
| 2)..... | | | | — | — | 22.64 |
| Calculated for.....C ₁₁ H ₁₅ N ₅ SO ₃ -C ₆ H ₃ N ₅ O ₇ | | 38.78 | | 3.42 | 21.29 | 6.08 |

25) Picrate of nicotinic acid :- The mother liquor from the preceding picrate (24) was concentrated to a small volume and a slight excess of picric acid was added. On cooling, the picrate of nicotinic acid crystallized out. Yield : 13.2g. M. P.=218° (Uncorr.)

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 4.95 | 0.681 | 0.695-2Vol% | 17 | 761 | 16.21 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % | |
|---|---------------|------------------------|-------------------------|--------|--------|-------|
| 1 | 8.10 | 12.37 | 1.88 | 41.65 | 2.58 | |
| Found/by Pregl's micro-analysis | | | | C% | H% | N% |
| 1)..... | | | | 41.65 | 2.58 | 16.21 |
| Calculated for.....C ₆ H ₄ NCO ₂ H-C ₆ H ₃ N ₅ O ₇ | | 41.19 | | 2.27 | 2.27 | 15.91 |

d) The filtrate from the precipitate (c) was treated with an excess of silver nitrate and baryta. The precipitate formed, was decomposed with hydrogen sulphide and filtered. The filtrate was evaporated in vacuum and precipitated again with phosphotungstic acid. A voluminous precipitate was decomposed with baryta water, and filtered. The filtrate freed from an excess of baryta, was evaporated in vacuo to a small volume and picric acid was added. A picrate of unknown base separated in yellow plates which were recrystallized from hot dilute alcohol. Yield : 9.2g.

24) Picrate of unknown base I. (C₃H₆N₂) :- Elongated thin plates with light yellow color, readily soluble in hot water but sparingly soluble in cold water, insoluble in ether, benzene etc., melts at 225°C (Uncorr.) followed by decomposition with evolution of gas. Dried at 100°C in vacuo and analysed;

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 4.27 | 0.8428 | 0.86-2Vol% | 17 | 761 | 23.22 |
| 2 | 4.20 | 0.8330 | 0.85-2Vol% | 17 | 765 | 23.49 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 3 | 7.78 | 10.74 | 2.07 | 37.65 | 2.96 |
| 4 | 7.41 | 10.21 | 2.03 | 37.58 | 3.04 |
| 5 | 8.03 | 10.91 | 2.06 | 37.05 | 2.85 |

c) Picric acid :-

| No. | Subst mg. | Picric acid mg. | Picric acid % |
|-----|--------------|--------------------|------------------|
| 1 | 200.0 | 153.06 | 76.53 |
| 2 | 260.0 | 200.1 | 76.96 |
| 3 | 500.0 | 382.7 | 76.54 |

| Found | C % | H % | N % | Picric acid.% |
|---|-------|------|-------|---------------|
| 1)..... | 37.65 | 2.96 | 23.22 | 76.53 |
| 2)..... | 37.58 | 3.04 | 23.49 | 76.96 |
| 3)..... | 37.05 | 2.85 | — | 76.59 |
| Calculated forC ₉ H ₆ N ₂ -C ₆ H ₃ N ₃ O ₇ | 36.12 | 3.01 | 23.41 | 76.59 |

Hydrochloride of the base :- The hydrochloride was prepared from the purified picrate by dissolving the latter in water and extracting the picric acid, liberated by the addition of hydrochloric acid, with ether. The aqueous solution was concentrated to a small volume and the hydrochloride was precipitated by adding absolute alcohol.

It crystallizes from dilute alcohol in colorless thick plates, readily soluble in water and sparingly soluble in absolute alcohol, melting at 262°C (Uncorr.) with decomposition. It gives white precipitate with phosphotungstic acid, while diazo-, biuret-, millon's and purin-reactions are negative.

Dried at 100°C in vacuo and analysed ;

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 5.74 | 1.166 | 1.19-2Vol% | 16 | 763 | 24.09 |
| 2 | 5.76 | 1.166 | 1.19-2Vol% | 16 | 763 | 24.00 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 3 | 7.14 | 8.61 | 4.44 | 32.89 | 6.91 |
| 4 | 7.31 | 8.78 | 4.44 | 32.77 | 6.75 |
| 5 | 8.41 | 10.16 | 4.88 | 32.95 | 6.45 |

| Found / by Pregl's micro-method | C % | H % | K % | CL% |
|--|-------|------|-------|-------|
| 1)..... | 32.89 | 6.91 | 24.09 | — |
| 2)..... | 32.77 | 6.75 | 24.00 | — |
| 3)..... | 32.95 | 6.45 | — | — |
| Calculated forC ₉ H ₆ N ₂ HCl | 33.96 | 6.60 | 26.42 | 33.33 |

Chloro-platinate of the base :- The chloro-platinate was prepared by adding the alcoholic solution of platinum-chloride to an aqueous solution of

the hydrochloride. Recrystallized from hot water, it forms reddish orange, thick plates, sparingly soluble in water, insoluble in alcohol, and decomposes at 280–285°C (Uncorr.) without melting.

Dried at 100°C in vacuo and analysed ;

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction. c.c. | Temp. C. | Atmosph.-press. mm. | N % |
|-----|---------------|--------------------|--------------------------|-------------|------------------------|--------|
| 1 | 6.75 | 0.552 | 0.563—2 Vol% | 15 | 764 | 9.74 |
| 2 | 6.27 | 0.529 | 0.54 —2 Vol% | 16 | 767 | 10.09 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | Pt mg. | C % | H % | Pt % |
|---|---------------|------------------------|-------------------------|-----------|--------|--------|---------|
| 3 | 9.05 | 4.47 | 2.29 | 3.17 | 13.47 | 2.81 | 35.19 |
| 4 | 8.92 | 4.43 | 2.11 | 3.09 | 13.54 | 2.63 | 34.65 |
| 5 | 8.59 | 4.13 | 2.11 | 2.96 | 13.08 | 2.73 | 34.46 |
| Found / by Pregl's micro-method | | | | | C % | H % | N % |
| 1)..... | | | | | 13.47 | 2.81 | 9.74 |
| 2)..... | | | | | 13.54 | 2.63 | 10.09 |
| 3)..... | | | | | 13.08 | 2.73 | — |
| Calculated for.....(C ₃ H ₆ N ₂) ₂ —H ₂ PtCl ₆ | | | | | 13.16 | 2.56 | 10.02 |
| | | | | | | | 35.65 |

From these results the free base is considered to be an amine C₃H₆N₂, differing in many respects from histidin, arginin, etc. Hoffa⁽¹⁾ once isolated an amine—C₃H₆N₂ "Anthracin"— with unknown structure. Whether the present amine is identical with anthracin or not must be investigated later on.

27) Unknown base II. C₆H₈N₂O :- The mother liquor from the above picrate (26) was concentrated further and treated with an excess of picric acid. On cooling, a new picrate separated out in a large quantity. It was filtered by suction and recrystallized from dilute alcohol. Yield : 60g.

It crystallizes in yellow, tetragonal thin plates or prisms, melting at 193°C (Uncorr.), readily soluble in hot water, alcohol and glacial acetic acid, but insoluble in benzene, ether etc.

Dried at 80°C in vacuo and analysed :-

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction c.c. | Temp. C. | Atmosph.-press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|------------------------|--------|
| 1 | 4.925 | 0.828 | 0.845—2 Vol% | 17 | 761 | 19.81 |
| 2 | 4.17 | 0.686 | 0.70 — " | 17 | 764 | 19.46 |
| 3 | 4.44 | 0.735 | 0.75 — " | 16.5 | 764 | 19.62 |
| 4* | 4.75 | 0.774 | 0.79 — " | 18 | 754 | 18.96 |
| 5* | 5.04 | 0.848 | 0.865— " | 18 | 753 | 19.55 |
| 6** | 4.58 | 0.750 | 0.765— " | 18 | 755 | 19.08 |
| 7** | 4.76 | 0.797 | 0.813— " | 18 | 754 | 19.27 |

(1) Hoffa, Sitzungsber. d. Phys-Med. Gesellschaft, Wurzberg. 1889, 96.

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|------|---------------|------------------------|-------------------------|--------|--------|
| 8 | 7.54 | 11.75 | 2.19 | 42.50 | 3.23 |
| 9 | 7.32 | 11.30 | 1.92 | 42.11 | 2.91 |
| 10 | 7.28 | 11.45 | 1.94 | 42.89 | 2.96 |
| 11** | 7.24 | 11.21 | 2.10 | 42.23 | 3.23 |

c) Picric acid :-

| No. | Subst. mg. | Picric acid mg. | Picric acid % |
|-----|---------------|--------------------|------------------|
| 12 | 500.0 | 326.0 | 65.20 |
| 13 | 500.0 | 325.8 | 65.16 |
| 14 | 200.0 | 130.7 | 65.35 |

| Found | C % | H % | N % | Picric acid.% |
|---|-------|------|-------|---------------|
| 1)..... | 42.50 | 3.23 | 19.81 | 65.20 |
| 2)..... | 42.11 | 2.91 | 19.46 | 65.16 |
| 3)..... | 42.89 | 2.96 | 19.62 | 65.35 |
| 4)* (Purified with glacial acetic acid) | — | — | 18.96 | — |
| 5)* (" ") | — | — | 19.55 | — |
| 6)**(Prepared from hydrochloride) | 42.23 | 3.22 | 19.08 | — |
| 7)**(" ") | — | — | 19.27 | — |
| Calculated forC ₆ H ₈ N ₂ O-C ₆ H ₈ N ₃ O ₆ | 40.79 | 3.11 | 19.83 | 64.88 |

Hydrochloride of the base :- The hydrochloride was prepared from the purified picrate and recrystallized from alcohol.

It forms colorless, thin prisms, readily soluble in water, sparingly soluble in absolute alcohol but insoluble in ether, benzene etc. and melts at 237°C(Uncorr.). It gives white precipitate either with phosphotungstic acid or with mercuric chloride. When heated, it gives the pyrrol-reaction, while biuret, millon's, and diazo reactions are negative. Dried at 100°C in vacuo and analysed;

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|------------------------|--------|
| 1 | 4.33 | 0.637 | 0.65—2Vol% | 16 | 767 | 17.54 |
| 2 | 4.50 | 0.666 | 0.68—2Vol% | 16 | 767 | 17.64 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|-----|---------------|------------------------|-------------------------|--------|--------|
| 3 | 7.63 | 13.34 | 3.80 | 47.68 | 5.35 |
| 4 | 7.53 | 13.42 | 3.68 | 48.61 | 5.51 |

| Found / by Pregl's micro-method | C % | H % | N % | Cl% |
|---|-------|------|-------|-------|
| 1)..... | 47.68 | 5.53 | 17.54 | — |
| 2)..... | 48.61 | 5.51 | 17.64 | — |
| Calculated forO ₆ H ₈ N ₂ O-HCl..... | 45.86 | 5.10 | 17.61 | 22.26 |

Chloro-platinate of the base :- The chloroplatinate was prepared by

adding an alcoholic solution of platinum chloride to the aqueous solution of the hydrochloride.

It crystallizes in deep orange, thick plates, sparingly soluble in water, and melts at 283°C (Uncorr.).

It was dried at 100°C in vacuo and analysed;

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|------------------------|--------|
| 1 | 6.46 | 0.451 | 0.46—2Vol% | 16 | 767 | 8.34 |
| 2 | 6.45 | 0.451 | 0.46—2Vol% | 17 | 767 | 8.36 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | Pt mg. | C % | H % | Pt % |
|-----|---------------|------------------------|-------------------------|-----------|--------|--------|---------|
| 3 | 8.80 | 7.35 | 2.04 | 2.61 | 22.75 | 2.58 | 29.69 |
| 4 | 8.27 | 6.97 | 2.06 | 2.47 | 22.93 | 2.77 | 29.87 |
| 5 | 8.78 | 7.42 | 1.97 | 2.64 | 23.05 | 2.52 | 30.07 |

Found / by Pregl's micro-method.

| | C % | H % | N % | Pt % |
|---------|-------|------|------|-------|
| 1)..... | 22.75 | 2.58 | 8.34 | 29.69 |
| 2)..... | 22.93 | 2.77 | 8.36 | 29.87 |
| 3)..... | 23.05 | 2.52 | — | 30.07 |

Calculated for(C₈H₈N₂O₂)₂H₂PtCl₆ ... 21.95 2.44 8.54 28.20

From these results, it is proper to conclude that the free base has the formula C₈H₈N₂O. Further studies on this compound will be reported later on.

e) The filtrate from the fraction (d) was treated with hydrochloric acid and sulphuric acid to remove the excess of silver and barium. A 50% aqueous solution of phosphotungstic acid was then added, after making the contents of sulphuric acid to 5% of the solution. A voluminous precipitate, thus formed, was decomposed with baryta water in usual way. The filtrate was evaporated to a small volume and picric acid was added.

28) Cholin picrate ;— On cooling the solution, cholin picrate separated in large prisms which were recrystallized from hot dilute alcohol. Yield : 42g.

It forms characteristic large yellow prisms, readily soluble in hot water and alcohol, melting at 249°C (Uncorr.). Dried at 100°C in vacuo and analysed.

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|------------------------|--------|
| 1 | 4.66 | 0.647 | 0.66—2Vol% | 17 | 760 | 16.34 |
| 2 | 4.21 | 0.578 | 0.59—2Vol% | 17 | 764 | 16.24 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|--|---------------|------------------------|-------------------------|--------|----------------------------|
| 3 | 8.74 | 12.06 | 4.13 | 37.63 | 5.25 |
| Found / by Pregl's micro-method. | | | | | C % |
| 1) | | | | 37.63 | 5.25 |
| 2) | | | | — | 16.24 |
| Calculated forC ₅ H ₅ NO ₂ -C ₆ H ₃ N ₃ O ₇ | | | | | 37.71 5.14 16.00 |

The analysis agrees with cholin picrate.

29) Picrate of nicotinic acid :- The mother liquor from cholin picrate was treated with a slight excess of picric acid. On cooling, the picrate of nicotinic acid separated out. It was recrystallised from dilute alcohol. Yield: 12g. Light yellow, thick plates, melting at 219°C (Uncorr.).

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction c.c. | Temp. C. | Atmosph.-Press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|------------------------|--------|
| 1 | 4.96 | 0.676 | 0.69—2Vol% | 17 | 760 | 16.04 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|--|---------------|------------------------|-------------------------|--------|----------------------------|
| 2 | 7.86 | 12.00 | 1.73 | 41.64 | 2.45 |
| 3 | 7.71 | 11.65 | 1.54 | 41.21 | 2.22 |
| Found / by Pregl's micro-method. | | | | | C % |
| 1) | | | | 41.64 | 2.45 |
| 2) | | | | 41.21 | 2.22 |
| Calculated forC ₅ H ₄ NCO ₂ H-C ₆ H ₃ N ₃ O ₇ | | | | | 41.19 2.27 15.91 |

[C] The filtrate from the phosphotungstic acid precipitate [B] was treated with baryta to remove an excess of phosphotungstic acid as well as sulphuric acid. The filtrate freed from baryta, was evaporated in vacuum to a small bulk and kept in a cool place. White spherical crystals of thymin first separated out.

30) Thymin :- The crude crystals were recrystallized from hot water. Yield : 9g.

It forms short, white needles, sparingly soluble in cold water, readily soluble in hot water, and melts at 318–321°C (Uncorr.). It gives diazo-reaction. With ammoniacal silver solution, it gives white precipitate but no precipitate either with phosphotungstic acid or with picric acid. Dried at 100°C in vacuo and analysed ;

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction c.c. | Temp. C. | Atmosph.-press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|------------------------|--------|
| 1 | 6.16 | 1.186 | 1.21 —2Vol% | 17 | 760 | 22.66 |
| 2 | 4.80 | 0.916 | 0.935— " | 17 | 764 | 22.58 |
| 3 | 4.75 | 0.931 | 0.95 — " | 18 | 750 | 22.87 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|---|---------------|------------------------|-------------------------|--------|--------|
| 4 | 7.77 | 13.21 | 3.15 | 46.37 | 4.51 |
| 5 | 7.83 | 13.23 | 3.09 | 46.08 | 4.38 |
| Found / by Pregl's micro-method. | | | | C % | H % |
| 1) | | | | 46.37 | 4.51 |
| 2) | | | | 46.08 | 4.38 |
| 3) | | | — | — | — |
| Calculated forC ₅ H ₆ O ₂ N ₂ | | | | 47.62 | 4.76 |
| | | | | | N % |
| | | | | | 22.66 |
| | | | | | 22.58 |
| | | | | | 22.87 |
| | | | | | 22.22 |

The analysis agrees with thymine (Methyldioxypyrimidin).

31) Leucin :— The filtrate from thymine gave, on further concentration white spherules of leucin which were recrystallized from dilute alcohol. Yield : 4.8g.

Colorless thin plates, readily soluble in hot water, melting at 288°C (Uncorr.) with decomposition. It forms a blue copper salt, and gives a violet coloration by heating with ninhydrin.

a) Nitrogen :-

| No. | Subst. mg. | Vol. of N. c.c. | Vol.-Correction c.c. | Temp. C. | Atmosph.-press. mm. | N % |
|-----|---------------|--------------------|-------------------------|-------------|------------------------|--------|
| 1 | 6.02 | 0.6125 | 0.625—2 Vol% " " " | 17 | 762 | 10.35 |
| 2 | 5.98 | 0.534 | 0.545— " " " | 18.5 | 756 | 10.40 |

b) Carbon and hydrogen :-

| No. | Subst. mg. | CO ₂ mg. | H ₂ O mg. | C % | H % |
|--|---------------|------------------------|-------------------------|--------|--------|
| 3 | 7.64 | 15.56 | 6.87 | 55.54 | 9.99 |
| Found / by Pregl's micro-method. | | | | C % | H % |
| 1) | | | | 55.54 | 9.99 |
| 2) | | | — | — | — |
| Calculated forC ₆ H ₁₃ O ₂ N..... | | | | 54.97 | 9.99 |
| | | | | | N % |
| | | | | | 10.35 |
| | | | | | 10.40 |
| | | | | | 10.69 |

SUMMARY

The above results are summarized in the following scheme :—

In conclusion, the author desires to express his gratitude to Prof. U. Suzuki, who has continually encouraged and guided whenever needed, so as this work has taken the course unimpeded.

(Tokyo, Apr. 10th. 1927.)

Beer-yeast (Press.) 30,000 Kg.

Alcohol-Extract. (Curative dose for Pigeon, 0.1g.)

Tannin.-P.P.t.

-Crystall. Fraction I. 180g.

Filt. -Crystal

Hgcl₂ -P.P.t.

Pt. -Crystall.

80% Alcohol -P.P.t.

120g. Fraction II

-P.P.t. -AgNO₃ + Ba(OH)₂

Filt. -Phosphotungstic acid

50% Acetone. -Filt.

Filt. -Filt.

Fraction III 130g.

Filt. -Crystal

Filt. -P.P.t.

Absolute Acetone. -P.P.t.

-Filt. -AgNO₃ + BaCO₃

P.P.t. -Basic Pb-acetate

Filt. -Filt.

Compounds isolated.

| | | | |
|--------------------------------|-------|---|-------|
| (1) Adenylthiomethyl-Pentose | 70.0 | (1) Adenin (C ₅ H ₅ N ₅) | 206.0 |
| Kalium Sulphate, | 0.6 | (2) Adenylthiomethyl- | |
| (2) Thiamino-acid | 10.5 | Pentose-(C ₁₁ H ₁₅ N ₅ SO ₃) | 480.8 |
| (3) Leucin | 2.8 | | |
| (4) Adenin | 0.15 | (3) Cholin (C ₅ H ₁₅ NO ₂) | 15.4 |
| (5) Hypoxanthin-Picrate | 1.0 | (4) Hypoxanthin | |
| (6) Adenin-nicotinate | 0.05 | (C ₅ H ₄ N ₄ O) | 31.8 |
| (7) Unknownbase II Picrate | 0.18 | | |
| (8) Adenylthiomethyl-Pentose | 0.18 | | |
| Picrate | 2.5 | (5) Leucin (C ₆ H ₁₃ NO ₂) | 25.8 |
| (9) Cholin-Picrate | 0.3 | (6) Nicotinic acid | |
| (10) Tyrosin | 10.5 | (C ₅ H ₄ NCO ₂ H) | 26.0 |
| (11) Leucin | 0.05 | | |
| (12) Adenylthiomethyl-Pentose | 80.0 | (7) Thioamino-acid | |
| Kalium Sulphate | 0.05 | (C ₂ H ₁₁ SNO ₂) | 0.6 |
| (13) Adenylthiomethyl-Pentose | 310.0 | (8) Thymin (C ₆ H ₆ O ₂ N ₂) | 9.0 |
| (14) Adenin | 78.0 | (9) Tyrosin (C ₉ H ₁₁ NO ₃) | 0.3 |
| (15) Adenin-Hypoxanthin | 60.0 | (10) Base I. (C ₃ H ₆ N ₂) | 2.0 |
| (16) Adenin-Picrate | 60.0 | (11) Base II (C ₆ H ₈ N ₂ O) | 21.0 |
| (17) Adenylthiomethyl-Pentose | 6.0 | | |
| Picrate | 9.0 | | |
| (18) Hypoxanthin-Picrate | 4.8 | | |
| (19) Adenin | 19.2 | | |
| (20) Adenin-nicotinate | 4.2 | | |
| (21) Nicotinic-acid Picrate | 6.0 | | |
| (22) Adenin | 18.6 | | |
| (23) Adenin-Nicotinate | 6.0 | | |
| (24) Adenyl-thiomethyl-Pentose | 30.0 | | |
| Picrate | 13.2 | | |
| (25) Nicotinic-acid Picrate | 7.2 | | |
| (26) Unknown base I Picrate | 60.0 | | |
| (27) Unknown base II Picrate | 42.0 | | |
| (28) Cholin Picrate | 12.0 | | |
| (29) Nicotinic-acid Picrate | 9.0 | | |
| (30) Thymin | 4.8 | | |
| (31) Leucin | 4.8 | | |

Resinous-Substance

P.P.t.

Crude Yeast-Oryzamin
(Curative dose for
(Pigeon 0.01g)

昭和二年八月七日印刷
昭和二年八月十日發行

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